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Height growth, wood density and molecular markers to distinguish five tree species of Dipterocarpaceae grown at same site

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Abstract

The dipterocarps are of economic and ecological significance. To determine if simple traits can be used to distinguish wood of five representative species (*Dipterocarpus kerrii, Hopea plagata, Parashorea malaanoman, Shorea almon* and *Shorea contorta*), we determined tree height, wood density and chloroplastic (cp) DNA restriction patterns as a molecular fingerprint. Wood was obtained from trees of the same age grown at the same plantation (Leyte, Philippines). Data were subjected to cluster analysis (tree height, wood density) and used for the construction of a phylogenetic tree (cpDNA-PCR). Comparison of the result of cluster analysis with the phylogeny of the taxa based on cpDNA variation showed similar differentiation patterns. *D. kerrii* and *H. plagata* were always clearly separated from the other three species. Cluster analyses on tree characters revealed a clear grouping of all samples according to species, except for *S. contorta*, which was partly mixed with the subcluster of *P. malaanoman*. Since the formation of clades obtained by cpDNA-PCR analysis of samples was similar to that found by cluster analysis, our data indicate that wood traits can be used for taxonomic purposes to distinguish distantly related tree species of the same family.

Keywords: Dipterocarpaceae; Cluster analysis; Phylogeny; cpDNA.

Introduction

Dipterocarpaceae form an important pantropical tree family comprising three subfamilies according to recent classifications (Ashton, 1982; Loñdono *et al.,* 1995; Morton, 1995) with 'Dipterocarpoideae', 'Monotoideae' and the 'Pakaraimoideae'. Many tropical forests in Asia are dominated by dipterocarps (Dipterocarpoideae) and can be regarded as hot spots of global biological diversity (Cao *et al.,* 2006). The subfamily Dipterocarpoideae is very species-rich and common in Asian evergreen and Monsoon forests. The Asian Dipterocarps have been classified into two tribes based on morphology, wood anatomy and cytology (Gottwald and Parameswaran, 1966 and Ashton, 1982). The tribe Dipterocarpeae comprising the genera *Dipterocarpus, Anisoptera, Cotylelobium, Upuna,* and *Vatica.* The tribe Shoreae includes the genera *Hopea, Parashorea,* and *Shorea* (Ashton, 1982).

Dipterocarps are the most important commercial timber species in Southeast Asia. Indonesia supplied more than 70% of the world's demand for plywood, principally from dipterocarp species (Chong and Achmadi, 1996). Consequently, the timber industries of many Southeast Asian countries critically depend on trees of the family of Dipterocarpaceae (Cao *et al.,* 2006). Shorea is the largest and economically most important genus in this family (Seibert, 1996). Mixed dipterocarp forests are the world's main source of hardwood timber (Whitemore, 1984), especially since the end of the Second World War.

Several authors (Ashton, 1982; Ella, 1993; Lomibao, 1973; Newman *et al.*, 1996a; b; Newman *et al.*, 1998) described growth and main features for the Dipterocarps. The main features of the five important timber wood dipterocarps have been compiled in Table I. They can mainly be distinguished by their bark colours and types, leaf shape and sometimes by fruits (*D. kerrii*). Since the presence of a large number of dipterocarp species makes them difficult to distinguish, many different species are assembled under one name. The

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species rich genus *Shorea,* which contains more than 100 species, is differentiated into only a few trade names in many tropical countries like Indonesia (white meranti, yellow meranti, dark red and light red meranti) and the Philippines (red lulan-Philippine red mahogany and white lulan- Philippine light red mahogany) (Finkeldey *et al.,* 2008; Newman *et al.,* 1996a;b).

However, reliable species identification may require molecular genetic analysis. Molecular genetic markers can also be used to identify the origin of wood since the manipulation of genetic traits is impossible and since environmental conditions have no impact on variation patterns (Finkeldey *et al.,* 2008). To date, the number of marker types and reference data are increasing (Finkeldey *et al.,* 2007). The use of molecular markers in phylogenetic research has been adopted because of the simplicity in obtaining large amounts of data and the higher reliability as compared to morphological data for constructing phylogenetic trees (Chase *et al.,* 1993). Phylogenetic analyses are helpful in understanding relationships among species or higher-level comparisons. They also provide many new insights into the origin and biogeography of different groups of plants (Strauss *et al.*, 1992). A number of methods have been applied to clarify the phylogeny of Dipterocarpaceae, e.g. by using restriction fragment length polymorphism (RFLP) markers and nucleotide sequences of chloroplast cp(DNA) genes (Dayanandan *et al.*, 1999; Indrioko *et al.*, 2006; Kajita *et al.*, 1998; Morton *et al.,* 1999; Tsumura *et al.,* 1996), random amplified polymorphic DNA (RAPD) markers (Rath *et al.*, 1998) or partial sequences of nuclear genes (Kamiya *et al.*, 2005).

Wood is the most versatile and widely used structural material to be used in both indoor and outdoor applications. While the forest area in industrialized nations increased during the last decade, considerable losses of forest cover in developing countries diminish the forested areas in all main regions of the tropics (FAO, 2001). In order to limit illegal timber trading, the import and sale of tropical timber is greatly promoted by a proof of an origin in industrialized countries. Wood identification plays an important role in this context.

The main objective of this study was to find out whether simple characteristics like tree height, wood density can be used as means to distinguish species and to clarify phylogenetic relationships among taxa. To investigate these questions, we determined tree height, wood density of five selected dipterocarps, *D. kerrii, H. plagata, P. malaanoman, S. almon* and *S. contorta.* These taxa were chosen because of their economic significance. The results of tree traits were compared with those obtained by molecular analysis of cpDNA restriction data for species identification.

Materials and methods

Field site and sampling

Samples were collected in a plantation on the island Leyte (Philippines). The geographic and climatic condition of the site is summarized in Table II. Stem samples of 5 Dipterocarpaceae species (five replicate trees of each) were collected from a plantation at the western foothills of Mt. Pangasugan, within the forest reserve of the Leyte State University. The area was hilly to some extent and samples were taken on the upper part of the slope. The trees were planted at the same time and had an age of 6 years at harvest. Only healthy trees with no apparent injury were used. Disks excised from each tree at breast height (1.3 m) were used for determination of wood density. Blocks were cut from the disks of cross sectional dimensions ranging from 7.3 cm^2 to 2.2 cm^2, because of the variation of the tree diameter. Blocks were about 3 cm in height. For each species five individual trees were used for sampling.

Wood density determination

Wood disks were used for density determination (excluding the bark). It was not possible to remove the pith because of the small disk size of *S. almon*. The volume of fresh wood samples was determined by Archimedes' principle (Hacke *et al.*, 2000). The samples were immersed in a water-filled tray, which was placed on a balance. Displacement weight was converted to sample volume by the formula: displacement mass (g) / 0.998 (g cm^{-3}), where 0.998 g cm^{-3} is the density of water at 20 ^oC. The dry mass of the wood samples was measured after drying for at least 7 days at 35 ^oC. Wood density was calculated as the ratio of dry weight to volume of fresh wood.

Chloroplast DNA analyses

The total DNA was extracted from about 1 cm^2 of silica gel dried leaf tissue using the DNeasy Plant Kit (Qiagen, GmbH, Hilden). DNA amount and quality was checked on a 0.8% agarose gel after staining with ethidium bromide (Sambrook *et al.,* 1989). DNA was diluted 1:20 prior to PCR amplification.

Table I. Main features (including some physical characteristics and properties) for the identification of the main timber wood dipterocarp species.

Species	Tree height (m)	Diameter at breast height (cm)	Buttress	Outer bark	Inner bark	Bark thickness (mm)	Main diagnostic features
Dipterocarpus kerrii	30	130	if present, blunt	thin, grey, scaly	pinkish brown, brittle	no information available	rather small, shinning few-nerved, long-petioled leaves with few secondary nerves, the fallen stipules silky inside and the rather globose fruits
Hopea plagata	55	180	prominent	deep fissured bark	no information available	8	very hard and durable wood, leaves small, usually elliptical, thinly leathery , secondary nerves on lower leaf surface prominent but slender, upper leaf surface drying brown, mature fruit with two longer and upper shorter wings, number of stamens 32-38
Parashorea malaanoman	>60	~200	prominent	brown to almost black but greyish when exposed to sunlight	tan coloured with whitish vertical bands beneath the grooves	8	large size of trees, grey or almost black broadly fissured bark, large buttress, the medium-sized elliptic leaf with steeply ascending secondary nerves, matched-sized splinters burn charcoal to partial ash
Shorea almon	70	160	long and string	dark brown, dark brown , hard, ~0.6 mm thick, shallowly fissured	reddish yellow turning to reddish brown on exposure, fibrous, ~1.2 mm thick	11	the very dark or almost black bole, and the medium-sized, thin, slightly boat shaped leaves with a dense pale brown momentum beneath, match-sized splinters burn greyish or brown ash
Shorea contorta	50	180	present	brown to nearly black or grey when exposed to sunlight, V-fissured with vertical white strips of lenticels in the fissures	brown to slightly inkish, stringy	12	lower leaf surface smooth to touch; secondary nerves on ower leaf surface stoutly prominent, petals white, match-sized splinters burn white ash

Table II. Description of the field site of sample collection

Site	Geographic coordinates	Temperature (°C) Mean annual	Precipitation (mm) Average annual	Precipitation (mm) during the wettest months	Precipitation (mm) during the hottest months
Island Leyte (Philippines)	9°55´ N-11°48´ N and 124°17´ E-125°18´ E	27.4	2586 mm	November to January about 290 mm	March to May about 95 to 133 mm

Source: A weather station of the Philippines Atmospheric, Geophysical and Astronomical Service Administration (PAGASA, 7 m a. s. l.), Langenberger (2003, 2006).

Five cpDNA gene/interspecific region, *rbc*L, *pet*B, *psa*A (Tsumura *et al.,* 1995) and *trn*LF (tRNA-Leu (UAA) - tRNA-Phe (GAA) (Taberlet *et al.*, 1991) were amplified by PCR and digested with a total of seven restriction enzymes (*Alu*I*, Cfo*I*, Hae*III*, Hin*fI*, Msp*I*, Rsa*I*, Taq*I, see (Indrioko *et al.*, 2006). PCR conditions were chosen according to Tsumura *et al.* (1996) and Taberlet *et al.* (1991) with slight modifications. The restriction site data were transformed into a binary matrix (0/1). Length variants of restriction fragments were coded as multistate characters. A total of 117 informative characters were used as input for PAUP (Phylogenetic Analysis Using Parsimony) version 4.0 for Macintosh (Swofford, 1998).

The restriction site data were used to calculate a Neighbor joining tree (Saitou and Nei, 1987) based on the mean number of pairwise character differences. Statistical support of the clades was tested by 1000 bootstrap replications.

Statistical analysis

Statistical analysis was performed with SAS (9.13 version, SAS Institute Inc. 2004, Cary, NC, USA) using analysis of variance (ANOVA), followed by Duncan's multiple range test. Data are indicated as means ± SD. Differences between parameter means were considered significant when the *P* - value of the ANOVA Duncan's multiple range test was less than 0.05. Different letters indicate significant difference. Cluster analysis was done by Ward's method using Euclidean square as distance metric with the programme Statgraphic Plus (Statistical Graphics Corporation, St. Louis, MO, USA). Tree height and wood density served as input data.

Results and discussion

Growth and wood characteristics

Significant differences in tree height and wood density were observed among the five dipterocarp tree species (Table III).

Table III. Plant material, height and wood density. Data indicate means (± SD, n= 5). Different letters in columns indicate significant differences at $P \leq 0.05$

Timber name[a]	Species	Tree height (m)	Density (g cm^{-3})
Yakal	*Hopea plagata* (Blanco) S. Vidal	9.6 ± 1.14 A	0.97 ± 0.01 A
Minyak	*Dipterocarpus kerrii* King Damar	8.6 ± 0.89 A	0.70 ± 0.06 B
White Lulan	*Shorea contorta* Vidal	9.8 ± 1.30 A	0.40 ± 0.02 D
White Lulan bagtikan	*Parashorea malaanoman* (Blanco) Merr	7.2 ± 0.84 B	0.45 ± 0.03 C
Meranti	*Shorea almon* Foxw	4.1 ± 0.55 C	0.38 ± 0.03 D

[a]Kamiya *et al.* (2005) and Wood Density Database

To determine if tree height and wood density were sufficient to permit grouping of these trees according to species, cluster analysis was conducted with these two parameters (Fig. 1). This analysis revealed two main subclusters, one for *H. plagata* and *D. kerrii* and the other for *P. malaanoman, S. almon* and *S. contorta. D. kerrii* and *H. plagata* were clearly separated in the second order sub-cluster. *S. almon* and *P. mlaanoman* were also found to form separate second order subclusters. Only one sample of *S. contorta* was mixed up with the *Parashorea* subcluster. These data show that the five even-aged species grown at the same site were distinguishable by simple tree characters such height and wood density. However, in practical terms, tree height will usually be strongly variable due to differences in age and growth environments and thus, will only be applicable under certain, limited conditions.

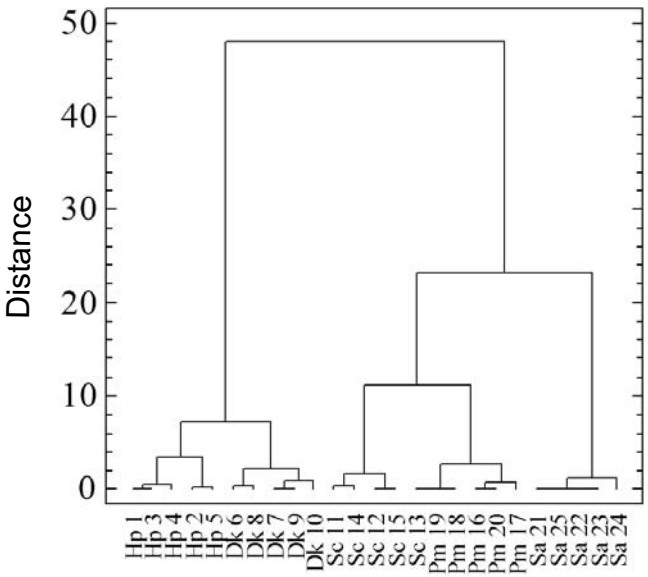

Fig. 1. Cluster analysis of five species of dipterocarps from a total of 25 different trees. Hp (*H. plagata*), Dk (*D. kerrii*), Pm (*P. malaanoman*), Sc (*S. contorta*) and Sa (*S. almon*) were grown at the same site. The dendrogramme was constructed by Ward's method (Euclidian distance) using tree height and density (From Tab. I)

Phylogenetic analysis with cpDNA restriction

To investigate the phylogenetic distance between the five species of this study, chloroplast DNA restriction analysis was performed (Fig. 2). The Neighbor Joining tree based on cpDNA restriction data (Fig. 2) showed a clear separation between *D. kerrii* (tribe Dipterocarpeae) and the tribe Shoreae. The Shoreae clade showed 100% bootstrap support and comprises, as expected, the species *H. plagata, S. contorta , S. almon* and *P. malaanoman.* While all samples of *Hopea plagata, Shorea almon*, and *Parashorea malaanoman* formed each a distinct group with high bootstrap support, of the three samples analysed of *S. contorta* two formed a sister group to *Hopea plagata*, and one *S. contorta* sample was mixed in the *P. malaanoman* clade.

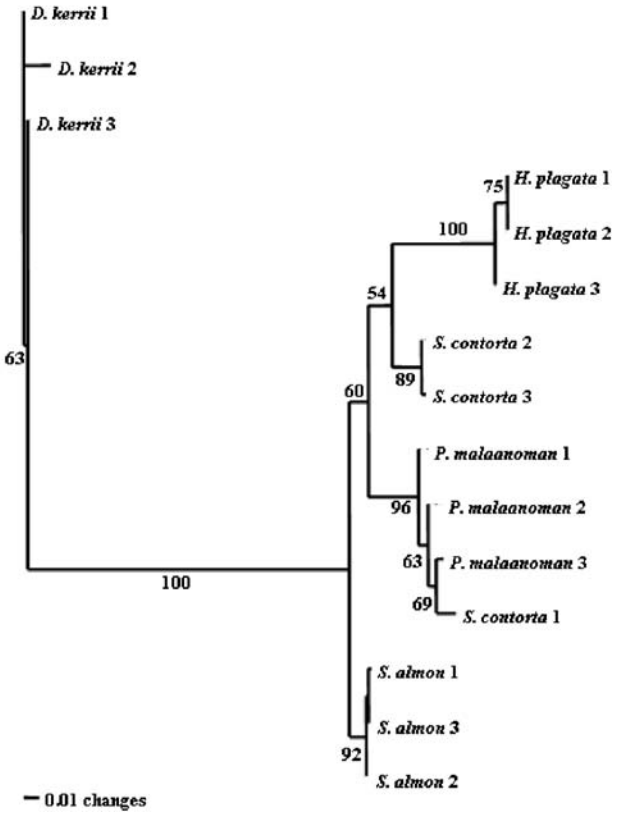

Fig. 2. Phylogenetic analysis of five dipterocarp species. The neighbor joining tree constructed based on modified data of Villarin (2007). Bootstrap values in percent from 1000 replicates are indicated above the nodes. The tree is unrooted and branch lengths are shown

Conclusion

Among the analysed tree species *H. plagata* belongs to heavy hardwood, *D. kerrii* to medium hardwood and the other three species belong to Philippine light red mahogany (Newman *et al.,* 1996b). This classification was also evident from the measured wood densities (Table III). According to publisheddata *H. plagata* and *D. kerrii* reach tree heights less

than those of *S. almon, S. contorta* and *P. malaanoman* (Table I). In contrast to this, among the trees of this plantation, *S. almon* was the smallest (Table III). In the wet tropical forests, the growth rate of sapling is associated with a number of factors like variation in leaf area and light level (Poorter, 2001; Sterck *et al.*, 1999). Growth rate increases linearly with light interception, although there is substantial unexplained variation in growth (King *et al.*, 2005). The dipterocarps vary greatly in growth rates and reach mature habit within 60 years under forest conditions; other tree species are usually shade tolerant and grow very slowly (Ashton, 1982). Because of the high plasticity in growth response, tree height is strongly variable and can only be used as a tree character if growth rates are known or if trees of the same age are growing at the same sites as in the present study. Using wood density and tree height as two simple traits, we have been able to group the trees of this study according to species with the exception of *S. contorta,* which was partly mixed with the cluster of *P. malaanoman* (Fig. 1). A similar grouping was achieved using molecular techniques (Fig. 2).

The phylogenetic tree obtained by cpDNA restriction (Fig. 2) indicates clear separation between the two tribes, Dipterocarpeae and Shoreae as well at the level of the genus (Hopea vs. Shorea). A number of studies were performed to unravel the relationship between *Shorea* and *Parashorea* species. Molecular phylogenetic studies (e.g. Cao *et al.*, 2006; Indrioko *et al.*, 2006; Tsumura *et al.,* 1996) and earlier morphological examinations (Symington, 1943) showed a close affinity between the genus *Parashorea* and the species-rich genus *Shorea*. Similar to our results on *S. contorta*, the diagnostic haplotypes of *Shorea fallax* fell into 5 different subclades throughout the clade of Red meranti in a phylogenetic tree derived from the nuclear gene *PgiC* suggesting interspecific hybridization or ancestral polymorphisms (Kamiya *et al.*, 2005). These examples underline that in some cases it is difficult to distinguish unambiguously some tropical species, even with current molecular techniques.

Our study of wood traits and growth parameters supports the close relationship between *Parashorea* and *Shorea* spp (Figs. 1). The differentiation patterns observed by means of cluster analysis (Fig. 1) reflect phylogenetic relationships among dipterocarps as assessed by PCR-RFLPs of cpDNA (Fig. 2).

The possibility to discriminate wood grown in same environment with a simple and rapid method is advantageous, if it is necessary to classify wood according to its provenience, for example for certification. Although a higher accuracy can be achieved by subjecting samples to genotyping by molecular methods, which are more laborious and expensive, this study shows that wood traits also have a potential to be used for taxonomic purposes and to clarify phylogenetic relationship within tree families.

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